

Control of Endometritis by Active Immunization Against Non-specific Pathogens in Cattle

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ABSTRACT

The present study revealed *E. coli*, *Staph. aureus*, *Strept. pyogenes* and *Strept. fecalis* as the most prevalent strains in case of endometritis in cattle. A formalized polyvalent vaccine was prepared from these non specific pathogens to control endometritis by active immunization. Results on rates of conception, bacterial isolation, the geometric mean titre of antibodies and histopathological findings before and after treatments were discussed. From these results, it was concluded that iso-immunization via the intrauterine infusion of blood plasma taken from actively immunized animals might be suggested to have a reasonable conception rate in cattle.

INTRODUCTION

It is known that the uterus of the cow frequently becomes contaminated with bacteria either at or immediately after calving. These organisms are normally eliminated spontaneously (1). But when they are not, particularly due to stress (2), endometritis takes place with an adverse effect on fertility (3, 4, 5). Many attempts had been adopted to treat endometritis in cattle. Some based on the intrauterine infusion of antibiotics (6, 7, 8, 9). Others depended on the intrauterine infusion of chemotherapeutic agents (10, 11, 12, 13). Some others recommended the intrauterine infusion of a biological matter like blood plasma (14, 15), colostrum (16) or honey-moon (17). The present study aimed at adopting a new method for controlling endometritis by active immunization against the non specific pathogens isolated from cows with endometritis and reproductive disorders.

MATERIAL AND METHODS

The present study was conducted on a herd of Holstein - Friesian cattle kept at the Italian- Egyptian project, El-Salhia, El-Sharkia Province. From a total of eight pleuriparous cows suffered from purulent endometritis, uterine swabs were taken under aseptic condition by using a sterile double sheathed swab- apparatus. These swabs were transferred to the lab on nutrient broth. For bacteriological examination, culturing on specific media, isolation and identification of microorganisms were carried out (18).

From the isolated strains, a Formalized polyvalent vaccine was prepared (19). The medium employed was a combination of Bains meduim No.(2) based on acid digest of casein and sternis pancreatic autodigest. It was sterilized by steaming freely for 15 mins, then by autoclaving at 121°C for 45 mins. After seeding with each of the isolated strains,

incubation was done at 37°C for 10-20 hs with aeration in a Bains vortex tank to prepare a dense culture. The latter was inactivated by adding formalin to a final concentration of 1% solution. Purity, safety and sterility of the formalized inactivated suspension of each strain were checked by injecting in susceptible mice, culturing in nutrient broth and nutrient agar. After 48 hs, absence of mortality in mice, turbidity of nutrient broth and colony appearance on nutrient agar approved its validity for application. Accordingly, the formalized inactivated suspensions of all strains were pooled and adjusted to concentration comparable with 25x-MacFrland density (approx. 3.25 X10⁶ bacilli/ml). This was used as a Formalized polyvalent vaccine after addition of phenol 1% as preservative.

A total of 35 cows kept in the project were diagnosed to have endometritis of 2nd - 3rd degrees as indicated by the rectal examination and the presence of mucopurulent - purulent vaginal discharge. These cows were divided randomly into three groups (Table, i).

Table (1) : Grouping of animals and system of treatment.

Group	No. of cases	Treatment	Dose ml	Route
1.Active immunization	13	Formalized polyvalent vaccine	10	intra muscular
2.Isoimmunization	12	Blood plasma taken from gr. (1) after one month of vaccination	100	Intra uterine
3.Nonimmunization	10	Blood plasma taken from the same non-vaccinated animal	100	Intra uterine

All groups were clinically examined to inspect the uterine conditions at the zero day and for two occasions with one month apart of treatment. Immediately before and after one month of treatment, uterine swabs were taken aseptically from all cows by means of the above mentioned apparatus. Thereby, the rate of isolation of strains used in vaccine preparation would be estimated before and after treatment.

Blood samples were collected by anti-puncturing before, 15 and 30 days after treatment for serology. The indirect hemagglutination test was conducted and the Geometric Mean titre (GMT) was estimated by using a table of antilogarithms (20). The mean titre simply equals the sum of the titration end points as tube or dilution number from any 2-fold dilution divided by the number of samples tested.

Uterine biopsy was taken by using sharp biopsy knife for histopathology. The biopsy was fixed in neutral buffered formalin, 10% solution, dehydrated, cleared, embedded in paraffin and sectioned 4-6µ in thickness, then stained with Hematoxylin- Eosin and described (21).

After two months from the last clinical examination and the first natural service, all cows were palpated rectally for pregnancy diagnosis.

Data obtained were tabulated and statistically analyzed to estimate rate of bacterial isolation, average GMT of antibodies and rate of conception as well as the significance between the different groups (22).

RESULTS

A) Before Treatment

On rectal examination, the affected uterus showed thick uterine wall, flabby texture and mucopurulent to purulent uterine contents discharged from the vagina on uterine palpation.

From the bacteriological examination, the most prevalent strains isolated from all uterine swabs taken from cows with purulent endometritis were *E. coli*, *Staph aureus*, *Strept. pyogenes* and *Strept faecalis* at rates of 30.30%, 22.28%, 27.72% and 19.65%, respectively (Table 2).

Table (2) The rate of bacterial isolation (%) before and after active -iso - and non - immunization against non specific pathogens

Isolated strains	Before treatment	After treatment		
		GR. (1)	GR. (2)	GR. (3)
<i>E. coli</i>	30.30	16.11	10.18	20.01
<i>Staph. aureus</i>	22.28	15.90	7.10	15.92
<i>Strept. pyogenes</i>	27.75	17.80	9.13	21.70
<i>Strept. faecalis</i>	19.65	7.10	2.90	12.40

The histopathological examination of the endometrial biopsy indicated mucopurulent and purulent endometritis. Acute mucopurulent endometritis was characterized by desquamation of the lining epithelium of uterine mucosa together with accumulation of homogenous basophilic mucus exudate infiltrated with polymorphnuclear and mononuclear cells (Fig.1). The submucosal blood vessels were dilated and engorged with blood; heavy submucosal mononuclear cellular infiltration was detected in some cases (Fig. 2). Chronic cases of mucopurulent endometritis revealed hyperplasia of the endometrial glands and periductal fibrosis (Fig. 3). Cystic dilatation of some endometrial glands with flattening of their lining epithelium were also observed (Fig. 4). Purulent endometritis revealed severe neutrophilic infiltration and destruction of the covering epithelium. The uterine glands showed accumulation of purulent exudate and large number of neutrophils in their lumen with destruction of the lining epithelium (Figs. 8, 9). Complete destruction of endometrial glands together with leucocytic cellular infiltration were also seen in some cases (Fig. 10).

By natural service, all affected cows suffered from failure of conception.

B) After Treatment

After two months of starting the treatment, cows in all treated groups relatively returned to the normal uterine condition as presented by the soft consistency and slight turgidity of the uterine wall, beside the nearly absence of the abnormal uterine contents.

Bacteriologically, the rate of bacterial isolation seemed to decrease significantly,

particularly in the second group of iso-immunization followed by that in the first group of active immunization comparing to that in the third group of non immunization (Table 2).

As shown from the indirect hemagglutination test (Table , 3), the average Geometric Mean Titre (GMT) for all isolated strains, seemed to increase significantly ($P < 0.01$) with the active immunized group from 25.55 ± 3.76 before to 159.82 ± 37.41 and 492.82 ± 67.40 after 15 and 30 days of treatment , respectively . With the iso-immunized group , the average GMT increased significantly ($P < 0.01$) only after 30 days of treatment (52.57 ± 5.74) when compared to before treatment (22.14 ± 6.14). However , with the non immunized group , the GMT seemed to be nearly the same before (24.14 ± 6.02) and after 30 days (23.14 ± 6.90) which appeared non significantly higher than that after 15 days (13.00 ± 2.83) of treatment (Table , 3).

Table (3) : The geometric mean titre (GMT) for the isolated strains indicated by the indirect hemagglutination test.

Treatment	Groups		
	Active-immunization	Iso-immunization	Non-immunization
No. of case	13	12	13
Before	25.55 ± 3.76^c	22.14 ± 6.14^b	24.14 ± 6.02^a
After			
-15 day	159.82 ± 34.14^b	24.00 ± 2.58^b	13.00 ± 2.83^a
-30 days	498.82 ± 62.40^a	52.57 ± 5.74^a	23.14 ± 6.90^a

Values with different letters within the same column were significant at least at $P < 0.05$.

Histopathologically, in case mucopurulent endometritis, the uterine mucosa appeared relatively normal with incomplete regeneration of the lining epithelium and subepithelial leucocytic cellular infiltration (Fig. 5) . Chronic cases appeared somewhat normal except mild desquamation of the lining epithelium and severe mononuclear cellular infiltration (Fig.6). Uterine glands showed slight periglandular fibrosis and mononuclear cellular infiltration (Fig. 7). In case of purulent endometritis, slight hyperplasia of the endometrial glands and mononuclear cellular infiltration were not observed (Fig. 11). Moreover , heavy mononuclear cellular infiltration and focal areas of fibrosis were also detected in few cases (Fig 12).

By natural service , the treated cows possessed conception rates of 30.77% , 33 and 20.00% for the active- , iso - and non-immunized groups , respectively (Table , 4)

Table (4) : The rate of conception for cows immunized or non immunized against non pathogenic causing endometritis

Treated groups	Total cases	Pregnant cases	Conception rate
Active - immunization	13	4	30.77%
Iso- immunization	12	4	33%
Non-immunization	10	2	20%

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- Fig. (1): Uterine mucosa showed accumulation of mucus exudate infiltrated with polymorphnuclear and mononuclear cells. H & E stain x 400 .
- Fig. (2): Endometrium showed submucosal mononuclear cellular infiltration. H & E stain x 400.
- Fig. (3): Uterus showed hyperplasia of the endometrial glands and periductal fibrosis . H & F stain x 200 .
- Fig. (4): Endometrium showed cystic dilatation of the endometrial glands with flattening of their lining epithelium . H & E stain x 400 .
- Fig. (5): Uterine mucosa showed regeneration of the lining epithelium and subepithelial leucocytic cellular infiltration. H & F stain x 200 .
- Fig. (6): Endometrium showed mild desquamation of the lining epithelium and severe mononuclear cellular infiltration. H & E stain x 200 .
- Fig. (7) : Uterine glands showed slight periglandular fibrosis and mononuclear cellular infiltration. H & E stain x 200 .
- Fig. (8) : Uterine glands showed accumulation of large number of neutrophils in their lumens with destruction of lining epithelium . H & E stain x 200 .
- Fig. (9) : High power of Fig. 8 showed accumulation of neutrophils in the lumen of endometrial glands . H & E stain x 400 .
- Fig.(10) : Uterine mucosa showed destruction of endometrial glands together with leucocytic cellular infiltration. H & E stain x 400 .
- Fig.(11) : Uterine mucosa showed slight hyperplasia of endometrial glands and mononuclear cellular infiltration. H & E stain x 200 .
- Fig.(12) : Endometrium showed heavy mononuclear cellular infiltration and focal fibrosis . H & E stain x100 .



Fig. (1)

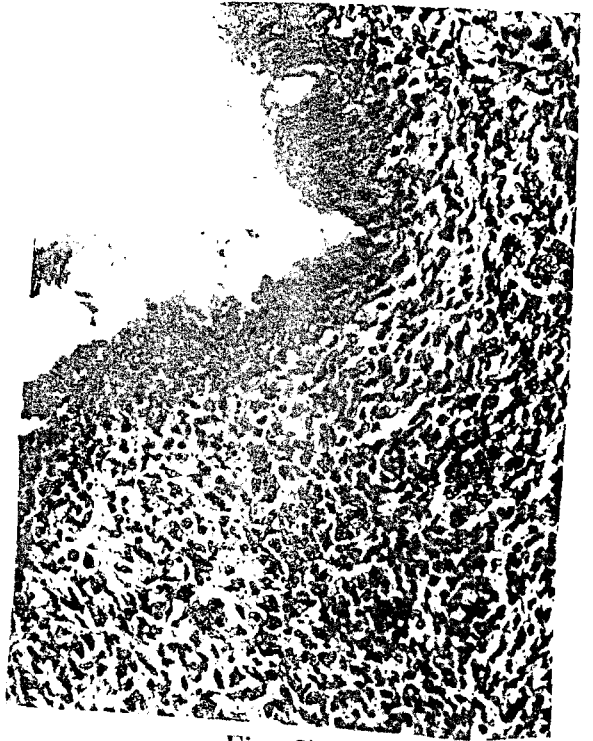


Fig. (2)



Fig. (3)

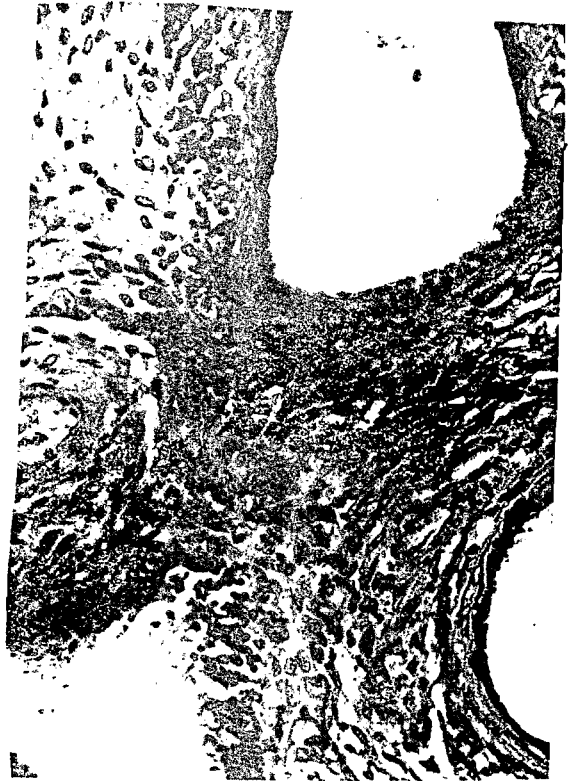


Fig. (4)



Fig. (6)



Fig. (7)



Fig. (8)



Fig. (9)

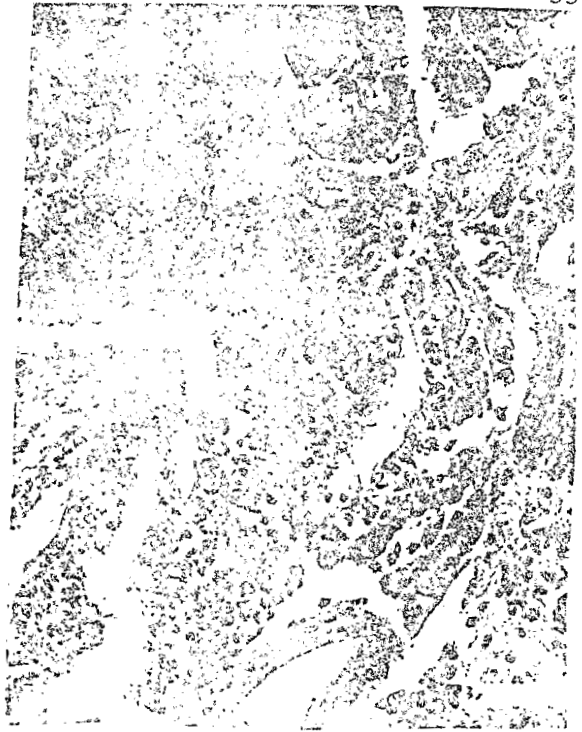


Fig. (10)



Fig. (11)



Fig. (12)

DISCUSSION

Infectious endometritis constitutes a major field problem hindering occurrence of pregnancy in bovine. Various workers reported the importance of non specific pathogens as the main cause of bovine endometritis (4, 23, 24, 25). From the present study, it has been noticed that *E. coli*, *Staph aureus*, *Strept. pyogenes* and *Strept. fecalis*, as non specific pathogens, were the most predominant strains could be isolated from the uterus in case of muco-purulent endometritis (Table 2). This finding came in agreement with some previous studies (3, 17, 26, 27). However, a highly significant difference in rate of isolation appeared between the different treated groups, comparing to that before treatment. The lowest rate came with the iso immunized group that had intra uterine infusion of blood plasma taken from the active immunized one. This finding assumed that the enriched immune bodies present in blood plasma after active immunization (Table, 3) are more effective locally via the intrauterine infusion than systematically via the blood circulation. That mean active immunization will not be sufficient to minimize the adverse effect of nonspecific bacteria on the uterus; otherwise local intrauterine infusion of blood plasma from actively immunized animal is suggested. This suggestion was emphasized by the results obtained with the conception rate (Table 1). However, lack of literatures are a great handicap to have a final agreement for active immunization against non specific pathogens. Nevertheless, an improvement was observed in the herd fertility status for animals vaccinated s. c. with 2 doses, 4 wks apart, of killed leptospira interrogans serovar hardjo vaccine as a result of higher overall conception rate and lower culling rates in these animals (28).

Because of the remarkable effect of iso-immunization on lowering the rate of bacterial reisolation and hence minimizing its drastic effect on the uterus, encouraging results were obtained by the histopathological study. These results appeared in or of healing the endometrial and glandular epithelial lining as well as rendering hyperplasia of the uterine mucosa, submucosal mononuclear infiltration and periglandular fibrosis of the uterine glands to the mild form. The histopathological findings in the present study came in

consistent with that demonstrated in previous reports (29, 30, 31, 32).

As shown from these findings, uterus, irrespective to type of treatment, did not return completely to its normal condition, an evidence to which the significant lower conception rate could be attributed. Further studies are necessary to justify accuracy of active immunization in controlling endometritis with special emphasis to recommended dose, time and route of injection, type and rate of infection.

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الملخص العربي

الوقاية من الالتهاب الرحمي بالتنشيط المناعي ضد مسببات الأمراض الغير تخصصية في الأبقار

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أظهرت الدراسة الحالية أن يكتريا الأيسريشا كولاي ، ساتاف أوريس ، ستروتوتويوجينيز سستوتوفيكال تعد العترات الأكثر انتشاراً في حالات الالتهابات الرحمية في الأبقار من هذه العترات امكن تحضير لقاح متعدد العترات ميت بالفورمالين ، تم الحصول على نتائج من حيث معدل الحمل ، العزل البكتيري ، المكافي الحسابي للاجسام المناعية والمشاهدات والمستويات لوجسة ، ومن هذه النتائج أمكن استنساخ أن المناعة المنقول عبر الحقن الرحمي بلازما الدم المأخوذة من حيوانات محصنة باللقاح قد يكون مقدما لحصول على معدل حمل معقول في الأبقار.